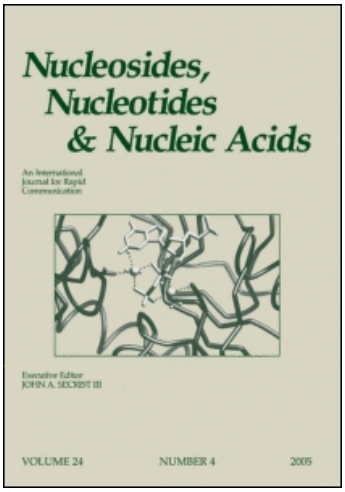

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9-(2-Deoxy-ß-D-xylofuranosyl)adenine and 1-(2-Deoxy-ß-D-xylofuranosyl)thymine: Phosphorylation and Stability

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9-(2-DEOXY-ß-D-XYLOFURANOSYL)ADENINE AND 1-(2-DEOXY-ß-D-XYLOFURANOSYL)THYMINE: PHOSPHORYLATION AND STABILITY

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ABSTRACT

Phosphorylation of 1-(2-deoxy-ß-D-xylofuranosyl)thymine (**1**) or 9-(2-deoxy-ß-D-xylofuranosyl)adenine (**3**) with phosphoryl chloride gives the cyclic 3',5'-phosphates (**2** and **4a**) but not the 5'-monophosphates **8a** or **8b**. The latter are obtained by phosphorylation of the 3'-O-benzoylated 2'-deoxy-ß-D-xylonucleosides (**7a**, **b**) and subsequent base-catalyzed removal of the benzoyl groups. Compound **3**, as the parent dA, depurinates in acidic medium, a reaction which is facilitated in the case of the N^6-benzoyl derivative **9b** and reduced after the introduction of an amidine protecting group. N-Glycosylic bond hydrolysis of 2'-deoxy-ß-D-xylofuranosyl nucleosides is enhanced by a factor of two compared to 2'-deoxy-ß-D-ribofuranosyl nucleosides.

INTRODUCTION

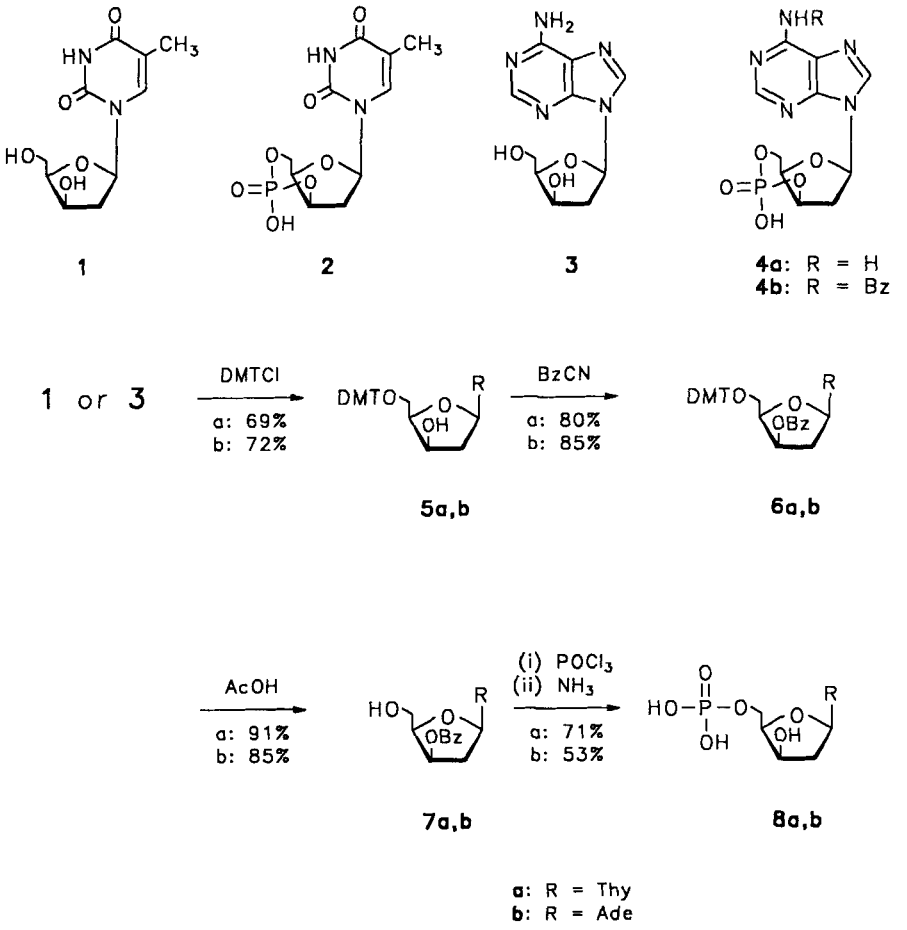
A common method for the preparation of nucleoside 5'-monophosphates is the reaction of unprotected nucleosides with phosphoryl or pyrophosphoryl chloride in trimethyl phosphate or acetonitrile [1,2]. Di- or triphosphates are obtained by condensation of 5'-monophosphates with inorganic ortho- or pyrophosphates in the presence of carbonyldiimidazole [3]. One pot reactions for the preparation of triphosphates have been described [4]. These procedures

have not only been used for regular nucleosides but also for the synthesis of base- and sugar-modified compounds [5,6]. In the case of 2'-deoxy-ß-D-xylofuranosyl nucleosides the situation is exceptional as the 3'-hydroxyl group is located at the same site as the 5'-OH function favouring cyclic 3',5'-phosphate formation [7,8]. In the following we report on the phosphorylation of 1-(2-deoxy-ß-D-xylofuranosyl)thymine (**1**) and 9-(2-deoxy-ß-D-xylofuranosyl)adenine (**3**) (systematic names see Experimental) under various reaction conditions. As hydrochloric acid is liberated during $POCl_3$ phosphorylation N-glycosylic bond hydrolysis can occur. Therefore, the stability of 2'-deoxy-ß-D-xylofuranosyl nucleosides against acid will be studied and the kinetic data will be compared with the parent 2'-deoxy-ß-D-ribofuranosyl nucleosides.

RESULTS AND DISCUSSION.

The treatment of 1-(2-deoxy-ß-D-xylofuranosyl)thymine (**1**) or 9-(2-deoxy-ß-D-xylofuranosyl)adenine (**3**) with phosphoryl chloride in trimethyl phosphate according to [1] did not result in the formation of 5'-phosphates. Instead, 3',5'-cyclic phosphates (**2** and **4a**) were formed as the only phosphorous-containing products. The cyclic phosphate **2** has already been obtained as a side product from 3'-deoxy-3'-iodothymidine triphosphate [9]. The formation of 1-(2-deoxy-ß-D-xylofuranosyl)uracil cyclic 3',5'-phosphate has been also reported [7]. In our case the yield of the adenosine derivative **4a** was lower than that of **2** probably of partial N-glycosylic bond hydrolysis.

In order to avoid N-glycosylic bond hydrolysis in the case of the xylo derivative **3** phosphorylation was performed in the presence of the proton sponge 2-tert-butylimino-2-diethylamino-1,3-dimethyl-perhydro-1,3,2-diazaphosphorine (BEMP) [10]. The reaction afforded the cyclic phosphate **4a** in an overall yield of 54%; no 5'-monophosphate was detected. The same occurred with 2'-deoxyxylothymidine (**1**). Compound **2** was isolated in 66% yield in the absence of the proton sponge. Also an alternative route using phosphoryl chloride and triazole [11] in acetonitrile was studied with 2'-deoxyxyloadenosine (**3**) and its N^6-benzoyl derivative **9b**. In the case of **3** the reaction failed, probably due to its low solubility.



SCHEME 1

The same reaction performed with N^6-benzoyl derivative **9b** gave **4b** as an intermediate. This was deprotected in aqueous ammonia to give the cyclic 3',5'-phosphate **4a** in 58% yield, identical with that obtained from **3**. The cyclic phosphates **2** and **4a** were characterized by ^1H-, ^{13}C-, and ^{31}P-NMR spectra as well as by electrophoretic mobilities and HPLC retention times (Table 1 and 2).

Compounds **2** and **4a** are resistant against enzymatic hydrolysis with alkaline phosphatase. Also the action of 50% acetic acid at 50°C (5 h) leaves compound **2** without change. Partial cleavage of the N-glycosylic bond is observed in the case of the adenine derivative **4a**. This side reaction resulted in a 10% loss of material (1.5 h; 50% after 5h) TLC: EtOAc/acetone/EtOH/water, 15:3:4:3.

The use of enzymatic phosphorylation would be an alternative route [7,12-14]. 9-(2-Deoxy-ß-D-xylofuranosyl)adenine monophosphate (**8b**) has already been prepared enzymatically from the nucleoside **3** by the action of a phosphotransferase isolated from carrots and p-nitrophenyl phosphate as the donor [12]. In order to synthesize 5'-monophosphates of 2'-deoxy-ß-D-xylofuranosyl nucleosides chemically without formation of cyclic phosphates, the 3'-O-benzoyl-protected derivatives **7a, b** were prepared. For the preparation of **7a, b** a similar method was used as reported for uridine derivative [7]. Compound **1** was converted into its 4,4'-dimethoxytrityl (DMT) derivative **5a**. The benzoyl group was introduced by the action of benzoyl cyanide in acetonitrile in the presence of triethyl amine resulting in **6a**; the subsequent treatment of the fully protected derivative **6a** with 80% acetic acid removed the 4,4'-dimethoxytrityl group, resulting in compound **7a**. The preparation of 3'-O-benzoyl-2-deoxy-ß-D-xylofuranosyl)adenine (**7b**) from the starting nucleoside **3** was analogously performed via compounds **5b** and **6b** as intermediates.

The reaction of **7a** or **7b** with phosphoryl chloride in trimethyl phosphate and the subsequent hydrolysis of intermediately formed dichlorophosphate was followed by alkaline hydrolysis of the benzoyl group. Regarding to the non-protected **3** its 3'-O-benzoyl derivative **7b** is more stable at the N-glycosylic bond. As a consequence, the phosphorylation of **7b** can be performed without a proton sponge, and adenine was not formed. The removal of the O-3' benzoyl group can be

Table 1. ^{13}C-NMR Chemical Shifts of 2'-Deoxy-ß-D-xylofuranosyl Nucleosides.[a]

Comp.	C(2)	C(4)	C(5)	C(6)	C(8)
1 [16]	150.6	137.3	108.7	163.9	
2	150.5	137.3	108.4	163.8	
3 [17]	148.6	152.3	119.0	156.2	140.1
4a	149.2	152.4	118.3	155.8	139.5
5b	148.7	152.3	119.1	156.2	139.9
6a	150.4	135.2	108.9	163.7	
6b	149.2	152.7	119.0	156.1	139.3
7a	150.4	135.7	109.0	163.8	
7b	149.1	152.6	119.1	156.1	138.5
8a	150.7	137.5	109.0	163.9	
8b	149.7	153.8	120.8	157.4	143.1

Comp.	C(1')	C(2')	C(3')	C(4')	C(5')	Me
1	83.5	40.8	68.7	84.9	59.6	12.6
2	83.2	40.5	75.1	76.4	63.9	12.3
3	82.5	40.3	69.3	85.3	60.0	
4a	82.0	40.6	75.6	76.8	64.9	
5b	83.0	40.6	69.6	83.7	63.2	
6a	84.0	38.6	73.0	80.9	61.2	12.2
6b	83.0[b]	37.4	73.2	81.0[b]	61.6	
7a	83.8[b]	38.6	73.0	83.1[b]	58.9	12.3
7b	83.0[b]	38.1	73.2	83.2[b]	59.3	
8a	83.3	40.6	68.4	82.9	61.3	12.5
8b	86.1	41.9	71.8	85.6	65.4	

[a] Measured in (D_6)DMSO at 296 K except **8b** (D_2O/CD_3OD 1:1); resonances of protective groups are not given. [b] Tentative.

Table 2. J(P,C)-Coupling Constants (Hz) of Compounds **2**, **4a**, **8a**, and **8b**.

Coupling	**2**	**4a**	**8a**	**8b**
^2J[P,C(3')]	5.3	5.0	0	0
^3J[P,C(4')]	5.8	5.2	5.3	7.6
^2J[P,C(5')]	5.2	5.0	4.0	4.8

performed either with aqueous ammonia at room temperature, or by the heating with sodium hydroxide in methanol/water. With respect to yield of the final 5'-monophosphates ammonia treatment is preferred. Purification of **8a,b** was carried out on a column of DEAE Sephadex using triethylammonium bicarbonate buffer as eluant.

The triethylammonium salts of **8a** and **8b** were eluted between 0.2-0.25 M. The overall yields based on **1** or **3** were 36% for **8a** and 28% for **8b**. Their structure was established by ^{1}H-, ^{13}C- and ^{31}P-NMR spectra (see Experimental and Tables 1 and 2). Both 5'-monophosphates migrate slower on TLC (2-propanol/25% aq. ammonia/H_2O, 7:1:1) than the cyclic phosphates **2** and **4a**, respectively. The opposite is found on electrophoresis.

The position of phosphorylation was derived from the phosphorous coupling constants of the ^{13}C-NMR spectra: the signals to C(4') and C(5') show long range coupling constants to phosphorous but not the signals of C-3'. Contrary to 5'-monophosphates, both cyclic phosphates **2** and **4a** show long range couplings of C(5'), C(4') and C(3') (Table 2).

We also tried to convert the 5'-monophosphate of 2'-deoxyxylothymidine (**8a**) into the corresponding 5'-triphosphate. For the synthesis the method of Hoard and Ott [3], i.e. the treatment of the nucleoside monophosphate with 1,1'-carbonyldiimidazole and subsequent reaction with tetrabutylammonium pyrophosphate was chosen. This method has been already successfully employed during the synthesis of *xylo*-ATP [15]. The major product of the reaction of **1** was the cyclic 3',5'-phosphate **2** (30% yield). However, other by-products were formed which were not identified. It is not clear whether the cyclic phosphate formation occurs directly by condensation of the 5'-monophosphate with the 3'-hydroxyl group (neighbour group participation) or via an intermediately formed 5'-triphosphate.

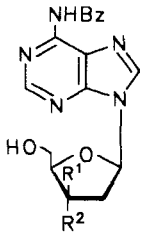
Next, we have studied the stability of 5'-monophosphates **8a, b** against hydrolysis by alkaline phosphatase. The reaction was carried out in 0.1 M Tris-HCl buffer (pH 8.3) at 37°C with identical amounts of alkaline phosphatase. The same conditions were used to monitor the dephosphorylation of dTMP and dAMP. Both xylophosphates were hydrolyzed at a slower rate (**8a**: $\tau_{1/2}$ = 6.5 min and **8b**: 5.0 min) compared to dTMP (2.3 min) and dAMP (1.3 min). These results are in agreement with those

reported in previous papers dealing with oligo(2'-deoxy-3'-xylofuranosylnucleotides). In those cases the phosphodiesterase hydrolysis of oligomers to the mixture of 5'-monophosphates was followed by dephosphorylation with alkaline phosphatase [16,17].

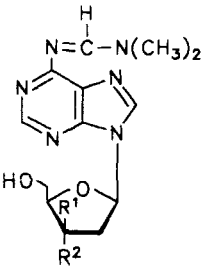
Furthermore, the stability of cyclic 3',5'-phosphates **2** and **4a** against enzymatic hydrolysis was assayed with 3',5'-cyclic nucleotide phosphodiesterase from bovine heart. Both compounds were found to be resistant against the enzyme. After 3 days of incubation (37°C) no trace of the corresponding 5'-monophosphates was formed. Under the same conditions (for details see Experimental) the hydrolysis of adenosine cyclic 3',5'-phosphate occurred with a half-life of 7.6 min yielding AMP.

The instability of the N-glycosylic bond of dA is a factor in oligonucleotide synthesis. Upon acid-catalysed detritylation depurination is the most severe side reaction. Also phosphorylation of 2'-deoxyadenosine performed in MeCN in the presence of small amounts of water/pyridine affords adenine in quantitative yield [2]. Moreover, it is known that N^6-benzoyl-2'-deoxyadenosine (**9a**) is even more sensitive towards hydrolysis than dA [18]. On the other hand it was reported that the N^6-dimethylaminomethylidene protecting group has a stabilizing effect [18]. With regard to this it was of interest to compare the stability of 9-(2-deoxy-ß-D-xylofuranosyl)adenine (**3**) with 2'-deoxyadenosine (dA) and also of the N^6-benzoyl- and N^6-dimethylaminomethylidene derivatives under acidic conditions.

The measurements were performed at 25°C in aq. HCl. The kinetic data were determined either UV-spectrophotometrically at the wavelength of maximal difference of UV absorbance or by HPLC-analysis (Table 3). In the case of HPLC analysis samples were taken at certain intervals of time and the reaction was quenched by neutralization. If *threo* and *erythro* nucleosides showed different retention times, e.g. dA and xA_d, hydrolysis experiments were carried out in a mixture of those nucleosides. In the case of compounds **10a** and **10b** kinetic data could not be determined by the change of the UV-spectra, as the main change of the spectrum appeared from the deprotection of the formamidine residue. According to the HPLC-analysis of the hydrolysis reaction of a dA/xA_d mixture (Fig. a) adenine is formed and in the case of **9b** bzA_d is



9a: R^1 = H; R^2 = OH
9b: R^1 = OH; R^2 = H



10a: R^1 = H; R^2 = OH
10b: R^1 = OH; R^2 = H

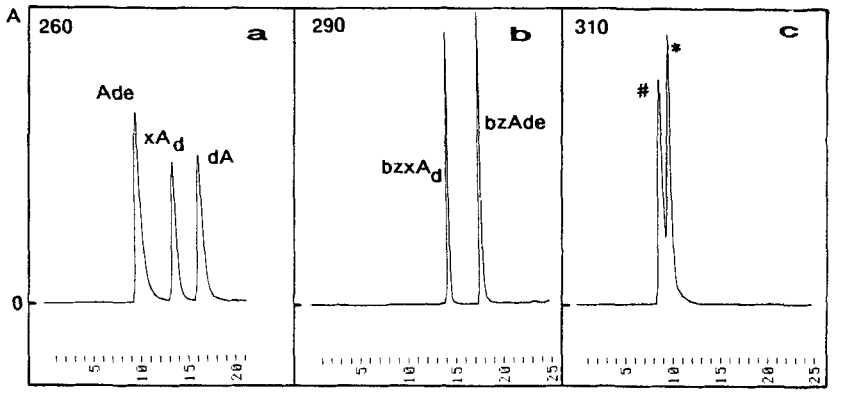


Figure. HPLC pattern of the reaction products obtained during proton-catalyzed hydrolysis of a dA/xA_d-mixture (a), **9b** ($bzxA_d$) (b), and N^6-dimethylaminomethylidene-2'-deoxyxyloadeosine (*) (c); dimethylaminomethylidene adenine (#). Conditions see Table 3 and Experimental.

Table 3. Hydrolytic Stability of 2'-Deoxyadenosine and 2'-Deoxyxyloadenosine Derivatives in aq. HCl at 25°C.

	HPLC[a] t_R [min] (λ_{max}; nm)	UV [nm]	τ 1/2 (HCl) [min] 0.1 N	0.2 N
dA	16 (260)	258	95[b]	36[c]
xA_d (3)	13 (260)	258	49[b]	18[c]
9a	15 (290)	291	34[c]; 35[b]	
9b	15 (290)		27[c];	
10a	10 (310)		3.2 h[c]	
10b	9 (310)		2 h[c]	

[a]For instrumentation see Experimental; 5% MeCN in 0.1 M triethylammonium acetate, pH 7.0 (A) and MeCN (B); in the case of dA and xA_d solvent A was used with a flow rate of 0.6 ml/min; for compound **10a** 8.5% MeCN and for **10b** 6% of MeCN in 0.1 M triethylammonium acetate were employed (1 ml/min); in the case of **9a, b** a gradient of B in A (0 - 30%) was used; flow rate 1 ml/min. [b]Determined by UV. [c]Determined by HPLC.

detected (Fig. b); dimethylaminomethylene adenine (#) is liberated during **10b** hydrolysis (Fig. c). If the HPLC analysis of the **10b** hydrolysis products is not monitored at 310 nm but at 260 nm adenine and 2'-deoxyxyloadenosine are also detected (data not shown). Therefore, the half-lifes of compounds **10b** and **10a** represent depurination together with deprotection of the base.

According to Table 3 it can be seen the 2'-deoxy-ß-D-xylofuranosyl nucleosides as well as their derivatives are always more labile than the parent 2'-deoxyribo compounds. Regarding acid-catalyzed deprotection during oligonucleotide synthesis the use of amidine-protected dA results in oligomers with apurinic sites and/or dA-residues without protecting groups. The dA-residues could then be modified, e.g. by the capping reagent. Regarding N-glycosylic bond hydrolysis the reduced stability of 2'-deoxy-ß-D-xylofuranosyl nucleosides may result from steric repulsion of the 3'-hydroxyl group

and the nucleobase, which facilitates the removal of the leaving group. A similar phenomenon has already been discussed in the case of the hydrolytically labile nucleoside wyosine [19]. Here, repulsion between the sugar moiety and the methyl group of the nucleobase is discussed.

EXPERIMENTAL

General. Elemental analyses were performed by Mikroanalytisches Labor Beller (Göttingen, Germany). NMR-spectra were measured on a AC 250 spectrometer (Bruker, Germany). Chemical shifts are in ppm relative to TMS as internal standard (^1H, ^{13}C) or to external 85% H_3PO_4 (^{31}P). ^{31}P NMR measurements were performed in D_2O/0.05 M Tris-HCl, pH 8.0, containing 100 mM Na_4EDTA. UV-spectra were recorded on a U 3200 spectrometer (Hitachi, Japan). Thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} plates (Merck, Germany). TLC scanning was carried out with Shimadzu TLC scanner (Japan). Column chromatography was performed on silica gel 60 (Merck, Germany). Reverse phase HPLC was carried out on a 4 x 250 mm RP 18 (7μm) Lichrosorb column (Merck) using a Merck-Hitachi HPLC pump (model 655 A-12) connected with a variable-wavelenth monitor (model 655-A), a controller (model L-5000), and an integrator (model D-2000). Electrophoresis was performed on silica gel glass plates (Sil G-25 with fluorescence indicator UV_{254}; Merck, Germany) for 2 h at 400 V and 50 mA, in 0.1 M sodium citrate (pH 6.5). Electrophoretic mobilities (E_{UP}) are related to uridine 5'-monophosphate. Alkaline phosphatase (EC 3.1.3.1., E. coli) and 3',5'-cyclic nucleotide phosphodiesterase (EC 3.1.4.17, bovine heart) were products of Boehringer, Mannheim (Germany). Compounds **1** (1-(2-deoxy-ß-D-*threo*-pentofuranosyl)thymine, **3** (9-(2-deoxy-ß-D-*threo*-pentofuranosyl)adenine, and **5a** were prepared according to [16, 17, 20]. The stability of compounds **2** and **4a** against proton-catalysed hydrolysis was assayed in 50% acetic acid at 50°C, the reaction course was monitored by TLC and electrophoresis.

1-(2-Deoxy-ß-D-*threo*-pentofuranosyl)thymine Cyclic 3',5'-Monophosphate, Triethylammonium (2).

The solution of compound **1** (59 mg, 0.24 mmol) in trimethyl phosphate (1 ml) was treated with $POCl_3$ (40 ul, 0.42 mmol) at 4°C for 5 h.

Triethylammonium bicarbonate (1M, pH 7.6, 10 ml) was added and after 1 h of stirring at room temperature the reaction mixture was evaporated. The residue was chromatographed on DEAE Sephadex (HCO_3^- form, column 30 x 2 cm). The chromatography was performed with water (400 ml) and then with a linear gradient of triethylammonium bicarbonate (0 - 0.3 M, 1200 ml). The cyclic phosphate was eluted at 0.2 M. Product-containing fractions were evaporated, coevaporated with water (3 x 50 ml) and the residue was chromatographed once more on DEAE Sephadex under the same conditions. The volatile salts were removed by repeated coevaporation with water, ethanol and acetone. 1394 A_{267} units (65%) of a colourless solid. TLC (2-propanol/25% aq. ammonia/water, 7:1:1): R_f 0.45; (EtOAc/acetone/EtOH/water, 15:3:4:3): R_f 0.09. HPLC (5% MeCN in 0.1 M triethylammonium acetate, pH 7.0; 0.6 ml/min): t_R 14.9 min. Electrophoresis: E_{UP} 0.64. UV (MeOH): λ_{max} 267 nm. ^{31}P-NMR (D_2O/0.1 M Tris-HCl buffer, 1:1): - 5.03 (d, J = 17.3). ^{1}H-NMR ((D_6)DMSO): 11.28 (s, NH); 10.79 (br, NH); 7.87 (s, H-C(6)); 6.12 (d, J = 7.9, H-C(1')); 4.69 (br, H-C(3')); 4.40 - 4.14 (m, CH_2 (5')); 3.64 (br, H-C(4')); 3.04 (q, CH_2 (Et)); 2.66 (m, H_α-C(2')); 1.96 (d, J = -16.2, H_β-C(2')); 1.76 (s, Me-C(5)); 1.20 (t, Me-(Et)).

9-(2-Deoxy-β-D-*threo*-pentofuranosyl)adenine Cyclic 3',5'-Monophosphate, Triethylammonium (4a).

Method A. The reaction of **3** (25 mg, 0.1 mmol) with $POCl_3$ in trimethyl phosphate was performed as described for compound **2**, but in the presence of BEMP (41 mg, 0.15 mmol). The reaction was completed after 12h. The work-up was the same as described for **2**. The final purification after chromatography on Sephadex was performed by preparative TLC using EtOAc/acetone/EtOH/water, 15:3:4:3 as solvent system: (R_f 0.13). 824 A_{260} units (53.8%) of colourless solid. TLC (2-propanol/25% aqueous ammonia/water, 7:1:1): R_f 0.54. HPLC (5% MeCN in 0.1M triethylammonium acetate, pH 7, 0.6 ml/min): t_R 15 min. Electrophoresis: E_{UP} 0.35. UV (MeOH): λ_{max} 260 nm. ^{31}P-NMR (D_2O/0.1 M Tris-HCl buffer, 1:1): - 5.11 (d, J = 19.4). ^{1}H-NMR ((D_6)DMSO): 9.97 (br, NH); 8.33 (s, H-C(8)); 8.15 (s, H-C(2)); 7.34 (s, NH_2); 6.40 (d, J = 7.8, H-C(1')); 4.63 (br, H-C(3')); 4.42 - 4.10 (m, CH_2 (5')); 3.94 (br, H-C(4')); 3.05 (q, CH_2); 2.85 (m, H_α-C(2')); 2.28 (d, J = -14.6, H_β-C(2')); 1.18 (t, Me).

Method B. The reaction was performed with the nucleoside **3** (20 mg, 0.08 mmol) as described for the method A but without addition of BEMP. After chromatography on DEAE Sephadex adenine (5 mg, 46%) was eluted at first with water and then compound **4a** with the buffer. Colourless solid (567 A_{260} units, 39%).

Method C. $POCl_3$ (20 µl, 0.21 mmol) was added to the solution of imidazole (86 mg, 1.26 mmol) in MeCN (0.5 ml). After stirring at room temperature for 30 min, the mixture was cooled to 0°C and the solution of **9b** (35 mg, 0.1 mmol) in MeCN/dimethylformamide (1:1; 1 ml) was added. The mixture was stored for 2 h at 4°C and then for 14 h at room temperature. After neutralization with 1 M aqueous triethylammonium bicarbonate (12 ml), the solution was stirred for 1 h and evaporated. The residue was treated with 25% aqueous ammonia (20 ml) for 16 h and evaporated. The purification of the product **4a** was carried out on DEAE Sephadex as described above. Residual deprotected starting nucleoside **3** was obtained during elution with water (24%), the product **4a** was eluted with a solution of 0.2 M triethylammonium bicarbonate. Colourless solid (843 A_{260} units, 57%).

1-[3-O-Benzoyl-2-deoxy-5-O-(4,4'-dimethoxytriphenylmethyl)-ß-D-*threo*-pentofuranosyl]thymine (6a).

Benzoyl cyanide (75 mg, 0.57 mmol) and triethyl amine (80 µl, 0.57 mmol) were added to the solution of **5a** (265 mg, 0.49 mmol) in MeCN (4 ml). After stirring at room temperature for 1 h, the reaction mixture was evaporated and the residue chromatographed on silica gel (column 2 x 17 cm) in CH_2Cl_2/acetone, 6:1 to give **6a** as white foam (255 mg, 81%). TLC (CH_2Cl_2/acetone, 6:1): R_f 0.56. ^{1}H-NMR ((D_6)DMSO): 11.32 (s, NH); 7.72 - 7.17 (m, 14 arom. H and H-C(6)); 6.80 - 6.61 (m, 4 arom. H); 6.18 (d, J = 5.7, H-C(1')); 5.68 (m, H-C(3')); 4.48 (m, H-C(4')); 3.69 (m, CH_2(5') and 2s, 2 MeO); 2.88 (m, H_α-C(2')); 2.26 (d, J = - 16.2, H_β-C(2')); 1.55 (s, Me). Anal. calcd. for $C_{38}H_{36}N_2O_8$ (648.71): C 70.36, H 5.59 N 4.32; found: C 70.31, H 5.70, N 4.36.

1-(3-O-Benzoyl-2-deoxy-ß-D-*threo*-pentofuranosyl)thymine (7a).

The solution of **6a** (200 mg, 0.31 mmol) in 80% acetic acid (3 ml) was stirred at room temperature for 15 min, then diluted with water (100

ml) and evaporated. The residue was coevaporated with water (50 ml), 2-propanol (50 ml) and MeOH (50 ml) and chromatographed on silica gel (column 2 x 12 cm) in CH_2Cl_2/MeOH, 95:5 to give **7a** as white foam (98 mg, 92%). TLC (CH_2Cl_2/MeOH, 95:5): R_f 0.30. ^{1}H-NMR ((D_6)DMSO): 11.29 (s, NH); 7.98 - 7.51 (m, 5 arom. H and H-C(6)); 6.14 (dd, J = 7.8, 2.3, H-C(1')); 5.54 (m, H-C(3')); 4.98 (t, OH-C(3')); 4.19 (m, H-C(4')); 3.82 (m, CH_2 (5')); 2.87 (m, H_α-C(2')); 2.20 (dd, J = -15.4, 2.0, H_β-C(2')); 1.69 (s, Me). Anal. calcd. for $C_{17}H_{18}N_2O_6$ (346.34): C 58.96, H 5.24, N 8.09; found: C 59.11, H 5.35 N 7.99.

1-(2-Deoxy-ß-D-*threo*-pentofuranosyl)thymine 5'-Monophosphate, Triethylammonium (8a).

Method A. The solution of **7a** (80 mg, 0.23 mmol) in $PO(OMe)_3$ (0.5 ml) was stored with $POCl_3$ (46 µl, 0.48 mmol) at 4°C for 8 h. The reaction mixture was poured into the cold 1 M aqueous triethylammonium bicarbonate (15 ml), stirred at room temperature for 2 h, evaporated and coevaporated with water (2 x 50 ml). The residue was treated with 25% aqueous ammonia (50 ml) for 3 h, evaporated and applied onto a column of DEAE Sephadex (HCO_3^- form, 2 x 30 cm). Elution was performed with water (600 ml) and then with a linear gradient of triethylammonium bicarbonate (0 - 0.3 M, 1200 ml). Phosphate containing fractions, eluted at 0.2 - 0.25 M, were evaporated, coevaporated with water and chromatographed once more on the column of DEAE Sephadex under the same conditions. Triethyammonium bicarbonate was removed by repeated coevaporation with water and EtOH yielding a colourless amorphous solid (1443 A_{267} units, 71%). TLC (EtOAc/acetone/EtOH/water, 15:3:4:3): R_f 0.06, (2-propanol/25% aq. ammonia/water, 7:1:1): R_f 0.10. HPLC (0.1 M Triethylammonium acetate/5% MeCN; 0.6 ml/min): t_R 8 min. Electrophoresis: E_{UP} 0.87. UV (H_2O): λ_{max} 267 nm. ^{31}P-NMR (D_2O/0.1 M Tris-HCl buffer): 1.36.

Method B. From **7a** (80 mg, 0.23 mmol) as described by method A. Except the removal of the benzoyl group was carried out with 0.1 M NaOH in 50% aqueous MeOH (15 ml) at 50°C for 2 h. The solution was neutralized with Dowex 50 (H^+ form), the ion exchanger was filtered off and the neutral solution evaporated. Further purification was performed as described above yielding **8a**. 1214 A_{276} units (60%).

9-[2-Deoxy-5-O-(4,4'-dimethoxytriphenylmethyl)-ß-D-*threo*-pentofuranosyl]adenine (5b).
Compound **3** (300 mg, 1.19 mmol) in pyridine (15 ml) was stirred with 4,4'-dimethoxytrityl chloride (508 mg, 1.5 mmol) and ethyldiisopropyl amine (255 µl, 1.5 mmol) for 12 h at room temperature. The solution was evaporated at 30°C, the residue coevaporated with toluene and chromatographed on the column of silica gel (column 4 x 11 cm) in CH_2Cl_2/acetone/Et_3N (40:40:3). White foam (476 mg, 72 %). TLC (CH_2Cl_2/acetone/Et_3N, 40:40:3): R_f 0.64. ^{1}H-NMR ((D_6)DMSO): 8.26 (s, H-C(8)); 8.16 (s, H-C(2)); 7.41 - 7.18 (m, 9 arom. H, NH_2); 6.84 - 6.77 (m, 4 arom. H); 6.35 (d, J = 8.2, H-C(1')); 5.94 (d, J = 5.2, OH-C(3')); 4.32 (m, H-C(3')); 4.19 (m, H-C(4')); 3.71 (s, 2 OMe); 3.23-3.02 (m, CH_2-(5'); 2.78 (m, H_α-C(2')); 2.29 (d, J = - 14.8, H_β- C(2')).

9-[3-O-Benzoyl-2-deoxy-5-O-(4,4'-dimethoxytriphenylmethyl)-ß-D-*threo*-pentofuranosyl]adenine (6b).
The solution of **5b** (400 mg, 0.72 mmol) in MeCN (4 ml) was stirred with benzoyl cyanide (105 mg, 0.8 mmol) and triethyl amine (130 µl, 0.93 mmol) for 3 h at room temperature. Another portion of benzoyl cyanide (25 mg, 0.19 mmol) and triethyl amine (25 µl) was added and the stirring was continued for 15 h. The solution was evaporated, the residue chromatographed on silica gel (column 2 x 15 cm) in CH_2Cl_2/acetone (1:2) to give a white foam (305 mg, 64%). TLC (CH_2Cl_2/acetone, 1:2): R_f 0.60. ^{1}H-NMR ((D_6)DMSO: 8.09 (s, H-C(8)); 8.07 (s, H-C(2)); 7.68-7.13 (m, 14 arom. H, NH_2); 6.75 - 6.69 (m, 4 arom. H); 6.41 (d, J = 5.2, H-C(1')); 5.82 (m, H-C(3')); 4.57 (m, H-C(4')); 3.69 (s, OMe); 3.67 (s, OMe); ca 3.3 - 3.2 (m, CH_2(5'); 3.05 (m, H_α -C(2')); 2.91 (d, J = - 14.3, H_β-C(2')).

9-(3-O-Benzoyl-2-deoxy-ß-D-*threo*-pentofuranosyl)adenine (7b).
Compound **6b** (260 mg, 0.40 mmol) was dissolved in 80% acetic acid (10 ml) and stirred at room temperature for 80 min. The solution was diluted with water (50 ml) and evaporated, the residue coevaporated with water (2 x 20 ml) and 2-propanol (20 ml). The crude product was applied on silica gel (column 2 x 12 cm) and chromatographed in CH_2Cl_2/MeOH 93:7. Colourless solid (120 mg, 85%). M.p. 166-168°C (2-propanol). TLC (CH_2Cl_2/MeOH, 93:7): R_f 0.30. ^{1}H-NMR ((D_6)DMSO): 8.28

(s, H-C(8)); 8.07 (s, H-C(2)); 7.85 - 7.47 (m, 5 arom. H); 7.27 (s, NH_2); 6.38 (d, J = 5.2, H-C(1')); 5.68 (m, H-C(3')); 5.01 (br, OH-C(5')); 4.33 (m, H-C(4')); 3.76 (m, H-C(5')); 3.02 (m, H_α-C(2')); 2.83 (d, J = -14.7, H_β-C(2')). Anal. calcd. for $C_{17}H_{17}N_5O_4$ (355.35): C 57.46, H 4.82, N 19.71; found: C 57.15, H 5.02, N 19.49.

9-(2-Deoxy-ß-D-*threo*-pentofuranosyl)adenine 5'-Phosphate, Triethylammonium (8b).

From compound **7b** (53 mg, 0.15 mmol) by the same route as described for **8a** (method A). For $POCl_3$ treatment the reaction time was 4 h. Amorphous solid (1222 A_{258} units, 53 %). It was dissolved in MeOH (ca 200 µl) and precipitated with ether (20 ml). The precipitate was collected by centrifugation and dried over phosphorous pentoxide. TLC (2-propanol/25% ammonia/water, 7:1:2): R_f 0.24, (EtOAc/acetone/EtOH/water, 15:3:4:3): R_f 0.06. HPLC (0.1 M Triethylammonium acetate/5% MeCN; 0.6 ml/min) t_R 13 min. Electrophoresis: E_{UP} 0.68. UV (MeOH): λ_{max} 259 nm. ^{31}P-NMR (D_2O/MeOD, 1:1): 2.94. ^{1}H-NMR (D_2O/MeOD, 1:1): 9.89 (s, H-C(8)); 9.71 (s, H-C(2)); 7.84 (d, J = 8.0, H-C(1')); 6.08 (m, H-C(3')); 5.77 - 5.5.61 (m, CH_2(5')); 5.01 (m, H-C(4')); 4.68 (q, CH_2); 4.48 (m, H_α-C(2')); 3.92 (d, H_β-C(2')); 2.74 (m, CH_3).

Hydrolysis of Nucleoside 5'-Phosphates with Alkaline Phosphatase.

The 5'-monophosphates **8a**, dTMP, and dAMP (0.2 A_{260} units, each) were dissolved in 0.1 M Tris-HCl buffer (pH 8.3, 200 µl) and incubated with alkaline phosphatase (2 µg) at 37°C. The reaction mixture was analysed by reverse phase HPLC in 0.1 M triethylammonium acetate containing 5% acetonitrile (0.6 ml/min). Quantification was made on the bases of peak areas. In the case **8b** the retention time was almost identical to that of the parent nucleoside **3** (13 min) and both substances were not separated. In this case **8b** (60 A_{260} units) was incubated with the enzyme (60 µg) in buffer solution (200 µl). The ratio of enzyme vs. substrate was the same as described above. The course of hydrolysis was monitored by TLC-scanning (2-propanol/25% ammonia/water, 7:1:2).

Hydrolysis of Nucleoside Cyclic 3',5'-Monophosphates using 3',5'-Cyclic Nucleotide Phosphodiesterase.

From the nucleoside cyclic 3',5'-monophosphates **2**, **4a** and 3',5'-cAMP (195 nmole/ml, each) in 0.1 M Tris-HCl (pH 7.5) containing 5 mM $MgCl_2$

samples (200 µl) were taken and a solution of the enzyme was added (4 µg). The mixture was incubated for at 37°C for 1 h in the case of 3',5'-cAMP and 3 days in the case of **2** and **4a**, respectively. The course of hydrolysis was monitored on reverse phase HPLC, as described above.

Kinetics of N-Glycosylic Bond Hydrolysis.
(A) HPLC: From an aq. nucleoside stock solution (300 µl, 3 mg) an aliquot (20 µl) was diluted with aq. HCl (1 ml). The mixture was storred at 25°C and samples were pipetted at intervals of time into aq. NaOH (40 µl) containing the equivalent amount of base in order to quench the reaction. Part of this solution was injected into the HPLC-apparatus. Retention times and gradients see Table 3.
(B) UV: Hydrolysis was followed at 25°C at the wavelength shown in Table 3.

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